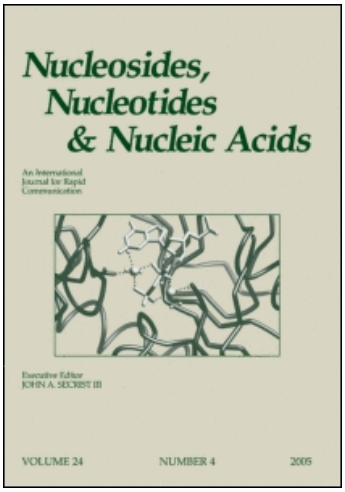

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Quantitative Measurements of Modified Thymine Residues in DNA Chains Formic Acid Hydrolysis

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QUANTITATIVE MEASUREMENTS OF MODIFIED THYMINE RESIDUES IN DNA CHAINS BY FORMIC ACID HYDROLYSIS

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Abstract

Mild formic acid hydrolysis at 90°C is a convenient method for releasing thymine modified residues which are formed upon gamma irradiation of DNA in oxygen-free solution from a polynucleotide chain backbone. The modified thymine residues are further isolated by chromatography and measured. The double helix prevents to some extent the attack of the thymine residue by the transient species OH, H and e^-_{aq} provided by water radiolysis.

Most of the biological effects observed when living cells are submitted to ionizing radiation are thought to be the result of the lesions which are induced in DNA.To determine the chemical structure of the DNA damage, preliminary studies were performed on free bases and free nucleosides [1, 2, 3, 4]. Presently, the major radioinduced modifications of the thymine residue upon gamma irradiation of DNA in oxygen free aqueous solutions have been well characterized at least for the monomeric compounds [5]. Recently, they have been found in DNA of human cells irradiated in in vivo conditions [6].

To be analysed and measured by chromatography and radioactive counting, the modified bases need to be liberated from the polymeric oligonucleotide chain. We have proposed formic acid hydrolysis in mild conditions as a method to rupture the N-glycosylic bond of the thymine radiation products which remain attached to the DNA chain after irradiation. This enabled us to determine the structure of the modified bases provided by the thymine residue when DNA is irradiated in oxygen-free solutions. We wish to report herein a kinetic study of their release

from the DNA chain backbone. We show that the method is accurate enough to measure their formation as a function of the applied dose.

RESULTS

DNA selectively ^{14}C-labeled on the thymine residues could be obtained by extraction from thymine auxotrophic E.coli mutant or by copy of a DNA template with labeled thymidine triphosphate and DNA polymerase. DNA was gamma irradiated in oxygen-free solutions. Ionizing radiation induced the release of a part of free thymine and modified thymine residues from the DNA chain backbone during irradiation time. This fraction was eliminated by dialysis against water. From the polymeric chain modified bases provided by the thymine residue could be released by mild formic acid hydrolysis (90°C). They were isolated by chromatography, identified as previously described and further measured by scintillation counting (fig. 1). Self-radiolysis due to the ^{14}C label did not significantly interfere with the radio-induced degradation due to the external gamma irradiation and could be neglected.

5,6-Dihydrothymine and 5,6-dihydro-5-hydroxythymine were readily liberated from the DNA chain and a maximum was attained after four hours. A slight decrease due to chain degradation by formic acid treatment was observed. The sum of cis and trans 5,6-dihydro-5,6-dihydroxythymine is given in the figure and a plateau is obtained at eight hours. 5,6-dihydro-6-hydroxy-thymine which is unstable in acidic conditions cannot be measured in this way.

A small percentage of unmodified free thymine residue is immediately released in the presence of formic acid which may be explained by a process of -elimination in which altered deoxyribose residues are involved. Thymine residues are further liberated slowly withtime.

The methodology has been applied to the measurement of the modified thymine residues versus the applied irradiation dose (fig. 2). The upward bend in the curve means that the 5,6-double bond of the pyrimidine ring is less available to radicals attack when DNA is double stranded. Irradiation induces the denaturation of the double helix and that accounts for the lag period which is observed.

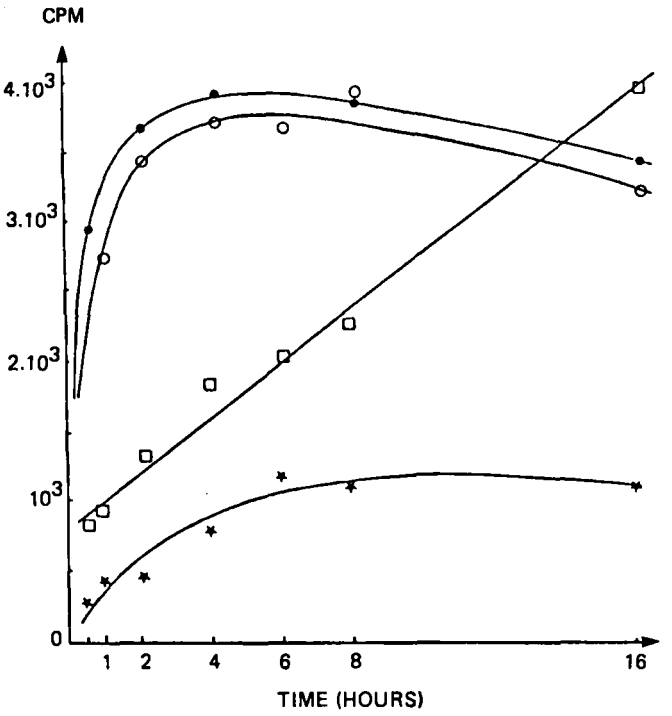

Fig. 1 : Release versus time of thymine and modified thymine residues from the ^{14}C-DNA gamma irradiated in deaerated aqueous solutions.
O 5,6-dihydro 5-hydroxy thymine ; thymine ; ★ cis and trans thymine glycols ; ● 5,6-dihydro thymine.

DISCUSSION

When free thymine was irradiated in deaerated solutions, the following G values were obtained : 5,6-dihydrothymine G = 0.27 ; 5,6-dihydro-5-hydroxy-thymine G = 0.040 ; 5,6-dihydro-5,6-dihydroxythymine G = 0.053 [7]. We obtain herein quite reasonable values for the thymine residue modifications which remain attached to the DNA chain : 5,6-dihydro-5,6-dihydroxythymine G = 0.005 to 0.006 ; 5,6-dihydrothymine and 5,6-dihydro-5,6-dihydroxythymine : G = 0.006 to 0.030.

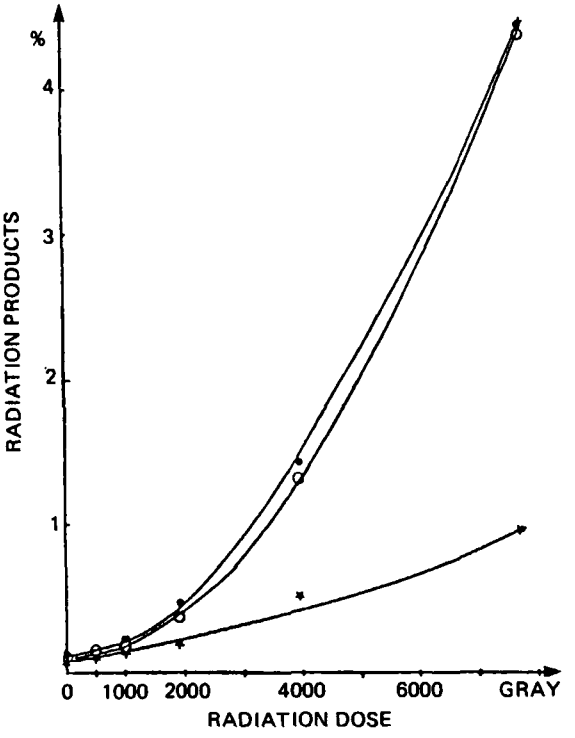

Fig. 2 : Formation of thymine radiation products inside the DNA chain versus the applied dose (percentage with respect to thymine residue)

● 5,6-dihydro thymine ; ★ cis and trans thymine glycols ; ○ 5,6-dihydro 5-hydroxy thymine.

The ratio of thymine radiation products has been related to the attack of e^-_{aq} OH and H radical at the C5 or C6 positions of the thymine ring and to the oxidizing or reducing properties of the transient pyrimidine radicals. Other effects at the DNA level have to be taken into account : steric hindrance, mobilities of the radicals inside the DNA chain structure and local molecular electrostatic potential in the DNA helix.

Inspection of the curve given in fig. 1 shows that formic acid hydrolysis is well adapted to the quantitative release of most of the

modified thymine residues. Coupling of this hydrolysis with a post-labeling procedure could be a method of choice in the measurement of radio-induced DNA thymine damage.

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